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The immune system and postmenopausal osteoporosis

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Abstract

In the last decay investigators have paid attention to the relation between immune system, estrogen deficiency and bone loss, some of the pathways have been clarified whereas others remain an unexplained challenge. This review summarizes the evidences that link immune cells, estrogen loss and osteoclast formation and activity.

Key words: osteoclast; T cells; B cells, cytokines; osteoporosis, immune system, menopause.

Introduction

The bone, hematopoietic, and immune system are in deep physical contact and share several common pathways; estrogens are well known regulators of bone metabolism and are also involved in the control of immune function. In the last decay the relation between estrogen deficiency, postmenopausal bone loss and immune system has become more evident.

Estrogen depletion increases osteoclasts (OCs) formation and activity, both directly and indirectly through immune cells. In particular estrogens modulates T and B cells activation and the production of cytokines that regulate bone turnover.

This review analyses how estrogen depletion influences bone metabolism through immune system.

Estrogen deficiency and OCs formation.

OCs precursors circulate within the mononuclear fraction of peripheral blood (D'Amelio et al., 2008; D'Amelio et al., 2010; D'Amelio et al., 2008; D'Amelio et al., 2010; D'Amico et al. 2013; Roato et al., 2008). This population acts not only as a reservoir for replenishing pre-OC pool in the bone marrow, but also as a potentially abundant source of pre-OCs that can be recruited into bone or joint tissue in response to reparative or pathological signals.

OC has been considered as an immune cell attracted in bone by stimulatory cytokines, expressed on accessory cells, and undergoing specific differentiation. OCs precursors increases during estrogen deficiency (D'Amelio et al., 2008) and in condition characterized by increased bone turnover as bone metastases (D'Amico et al. 2013;

Roato et al., 2007) or inflammatory diseases (Oostlander et al., 2012; Roato et al., 2007; Xue et al., 2012).

Estrogens act on OCs formation and activity both directly and indirectly. OCs express estrogen receptor (ER), estrogen binds with ER and biologic response is initiated as a result of a conformational change of the receptor, leading to gene transcription through specific estrogen response elements on target gene promoters (Ascenzi et al., 2006; Nilsson et al., 2001). These events result in increased or decreased mRNA levels associated with protein production (Smith et al., 2004; Tsai et al., 1994). In the non-classical transcription pathway, the estrogen-ER dimer co-activate with other transcription factors (TFs) e.g. AP-1, NF- κ B and SP-1, and binds non-ERE site to modulate gene expression by stimulating the general transcriptional machinery (Acconcia et al., 2006; Safe, 2001). Besides classical and non-classical nuclear pathways, an alternative non-genomic signalling is initiated by membrane bound receptors ERs and GPR30 acting as an acute response phase within seconds or minutes in bone cells (Kelly and Levin, 2001; Levin, 2002). The stimulation of ER depresses osteoclast catabolic activities.

Estrogen depletion up regulates OCs also indirectly through up-regulation of pro-osteoclastogenic cytokines produced by bone marrow cells (D'Amelio et al., 2011), osteoblast (Udagawa et al., 1999) and immune cells (D'Amelio et al., 2008; Onal et al., 2012; Qian et al., 2003; Roggia et al., 2001;).

Estrogen deficiency and cytokines.

Estrogen loss increases bone resorption acting through cytokine-driven increases in OCs formation; the minimal essential cytokines required for OCs formation are Macrophage Colony Stimulating Factor (M-CSF) and Receptor Activator of NF κ B Ligand (RANKL) (Hofbauer et al., 2000; Kong et al., 1999; Suda et al., 1999;).

RANKL is a member of the TNF super family that is present as both a trans-membrane molecule and a secreted form; it binds to its physiologic receptor RANK, which is expressed on the surface of osteoclast lineage cells. Its action is opposed by osteoprotegerin (OPG), a neutralizing soluble decoy receptor, produced by marrow stromal cells and OB (Hofbauer et al., 2004). The unbalance between RANKL and OPG has been indicated as the pivotal mechanism responsible for estrogen deficiency bone loss (Eghbali-Fatourehchi et al., 2003; Hofbauer et al., 2000).

M-CSF induces the proliferation of OCs precursors, the differentiation of more mature OCs, the fusion and increases the survival of mature OCs. RANKL promotes the differentiation of OC precursors into fully mature multinucleated OCs and stimulates the capacity of mature OCs to resorb bone.

A novel cytokine responsible for RANKL independent osteoclastogenesis has been recently identified and named Secreted Osteoclastogenic Factor of Activated T cells (SOFAT); this molecule promotes the differentiation of OCs precursors into bone resorbing OCs with a RANKL-independent mechanism (Rifas and Weitzmann 2009).

SOFAT was found to be derived from an unusual mRNA splice variant coded by the threonine synthase-like 2 gene homolog, and has no homology to any other known cytokine. This cytokine induces both osteoblastic IL-6 production and functional OCs formation in the absence of RANKL, it is not neutralized by OPG. The demonstration that SOFAT is a potent inducer of IL-6 production by OBs suggests that it could play a significant role in the local inflammatory response, and also could exacerbate bone destruction in rheumatoid arthritis, indirectly through multiple IL-6-mediated events (Rifas and Weitzmann 2009).

Additional inflammatory cytokines are responsible for the up-regulation of OC formation observed during estrogen deficiency; some of these molecules have a well-established

role in osteoclastogenesis and bone loss, while others have not. Among these molecules the most involved in estrogen deficiency bone loss appears to be $\text{TNF}\alpha$, IL-1, IL-7, IL-6 and IL-17.

$\text{TNF}\alpha$ enhances OCs formation by up-regulating stromal cell production of RANKL and M-CSF, and by increasing the responsiveness of OCs precursors to RANKL (Hotokezaka et al., 2007; Roato et al., 2006).

A key role of T cell-produced $\text{TNF}\alpha$ has been demonstrated in post-menopausal osteoporosis (D'Amelio et al., 2008) and in other condition as rheumatoid arthritis (Binder et al., 2013; Diarra et al., 2007; Takahata et al., 2012), multiple myeloma (Colucci et al., 2004; Kawano et al., 2011), and bone metastasis (Das Roy et al., 2009; Roato et al., 2005; Roato et al., 2008). The effect of $\text{TNF}\alpha$ on osteoclastogenesis is up-regulated by IL-1 (Wei et al., 2005), this cytokine enhances RANKL expression by bone marrow stromal cells and directly promotes OCs differentiation. In fact, treatment with IL-1 receptor antagonist decreases OCs formation and bone resorption in post-menopausal osteoporosis (Charatcharoenwitthaya et al., 2007).

Another cytokine involved in the regulation of immune system and in bone turnover is IL-7, this cytokine is able to enhance B and T cell number and reactivity to antigenic stimulus (Komschlies et al., 1994; Mackall et al., 2001) and have a controversial role in bone metabolism, it's mechanisms of action are only now beginning to be elucidated.

Some studies have demonstrated that IL-7 promotes osteoclastogenesis by up-regulating T cell-derived osteoclastogenic cytokines, including RANKL (Giuliani et al., 2002; Roato et al., 2008; Toraldo et al., 2003) and that the production is up-regulated by estrogen deficiency. *In vivo* IL-7 blockade suppresses T cell expansion and $\text{TNF}\alpha$ and $\text{IFN}\gamma$ production, preventing bone loss due to estrogen deprivation (Ryan et al., 2005; Weitzmann et al., 2002).

Post-menopausal bone loss is also associated with an increase in IL-6 level, activation of the signalling pathway mediated by glycoprotein (gp)-130 by IL-6 and its soluble receptor is historically known as a pivotal mechanism for the regulation of osteoclastogenesis (Zheng et al., 1997). IL-6 is not essential for bone resorption, however, IL6KO mice are protected against ovariectomy induced bone loss, and this finding, together with the observation of increased level of IL-6 after menopause in women, may suggest a peculiar role for IL-6 in bone loss due to estrogen deprivation. IL-6 was also shown to be increased in other diseases associated with increased OCs formation and activity such as Paget's disease of bone, multiple myeloma, rheumatoid arthritis and renal osteodystrophy.

In the recent year a role in osteoclastogenesis has been postulated for IL-17, this cytokine is mainly produced by human T helper 17 cell (Th17) (Yao et al., 1995). This cytokine plays a crucial role in inflammation and the development of autoimmune diseases such as rheumatoid arthritis; however, its mechanism of action in the development of bone erosions, especially in relation to other known key cytokines such as IL-1, $\text{TNF}\alpha$ and RANKL remains unclear. IL-17 has been suggested to be involved in the up-regulation of OCs formation in inflammation by increasing the release of RANKL, which may synergise with IL-1 and $\text{TNF}\alpha$ (Lubberts et al., 2005). Recently it has been suggested a role for IL-17 also in estrogen deficiency induced OCs formation; in ovariectomized mice there is an increase in Th17 cells with increased production of IL-17, this cytokine directly stimulated OCs differentiation and this effect is reversed by estrogens (Abdul et al., 2012).

One of the stimuli to IL-17 production is IL-23 produced by activated dendritic cells and macrophages. IL-23 drives the T helper 1 response, and is implicated in autoimmune diseases; hence; it has been suggested that the IL-23/IL-17 axis is critical for controlling inflammatory bone loss. However, in contrast to IL-17-deficient mice, IL-23 knockout mice are completely protected from bone and joint destruction in the collagen-induced arthritis model, indicating that the IL-23-induced bone loss may not be entirely mediated by IL-17,

and raising the question whether IL-23 can directly stimulate OCs. Recent work supports this hypothesis suggesting that IL-23 promotes OCs formation (Adamopoulos et al., 2011; Chen et al., 2008; Hu et al., 2013; Yago et al., 2007). Other recent in vivo studies suggest that IL-23 inhibits OC formation via T cells (Quinn et al., 2008). In physiological conditions (unlike inflammatory conditions), IL-23 favours higher bone mass in long bones by limiting resorption of immature bone forming below the growth plate (Quinn et al., 2008). These contrasting data suggest different roles of this cytokine in the control of physiological or inflammatory bone turnover.

In summary bone homeostasis is regulated by a large number of cytokines which exerts complex and overlapping effects on all bone cells.

Estrogen deficiency, T cells and the regulation of bone turnover.

Estrogens have a well-established role in the regulation of immune function, data on animals and humans demonstrated that both cellular and humoral immune responses are enhanced by estrogens (d'Elia et al., 2008; Kumru et al., 2004; Porter et al., 2001). Despite of some inverse reports (Anginot et al., 2007; Lee et al., 2006), the main body of literature firmly supports the essential role of activated T cells in regulating bone loss induced by estrogen deficiency (Abdul et al., 2012; Cenci et al., 2000; Cenci et al., 2003; D'Amelio et al., 2008; d'Elia et al., 2008; Eghbali-Fatourehchi et al., Gao et al., 2007; Grewal and Flavell, 1996; Kumru et al., 2004; Li et al., 2010; Porter et al., 2001; 2003; Rogers and Eastell, 2001; Roggia et al., 2001; Zheng et al., 1997).

Years ago some studies demonstrated, for the first time, in animal model the lack of bone loss after ovariectomy in absence of T cells and that bone loss is restored by T cells transfer (Cenci et al., 2000; Roggia et al., 2001).

In humans we have demonstrated that osteoclastogenesis from peripheral blood precursors occurs only in the presence of T cells and that T cells are more active than in healthy post- and pre-menopausal controls: this implies their greater ability to produce RANKL and $\text{TNF}\alpha$, thus inducing OCs formation and activity (D'Amelio et al., 2008). We have also shown that during menopause T cells are less prone to immune stimulation as respect to pre-menopausal healthy women. Moreover hormone replacement therapy decreases osteoclastogenic cytokine production in postmenopausal women (Rogers and Eastell, 2001).

In the bone marrow, ovariectomy promotes T cell activation by increasing antigen presentation by macrophages and dendritic cells (Adamski et al., 2004; Cenci et al., 2003). Estrogen loss also affects peripheral T cells by acting on thymus output of T cells into peripheral blood and its role in the expansion of bone marrow T cell pool (Ryan et al., 2005).

The increase in TNF produced by T cells during estrogen deficiency is explained by the increased expression of Class II TransActivator (CIITA), a transcriptional co-activator acting on MHCII promoter, with the final effect of up-regulation of expression of MHCII on macrophages (Cenci et al., 2003; Kim et al., 2010; Mueller et al., 2005), this subsequently expands the proliferation and lifespan of bone marrow T cells (Cenci et al., 2003; Gao et al., 2007).

RANKL-expression on lymphocytes and marrow stromal cells is significantly elevated during estrogen deficiency in humans and correlates directly with increases in bone resorption markers and inversely with serum estrogen levels (Eghbali-Fatourehchi et al., 2003).

A critical additional mechanism by which T cells deregulate bone homeostasis in ovariectomized mice is through CD40L-mediated cross-talk between T cells and stromal cells, which results in enhanced osteoclastogenesis. The CD40/CD40L system is crucial

for T-cell activation and several functions of the immune system. It promotes macrophage activation and differentiation, Antibody isotype switching, and the adequate organization of immunological memory in B cells (Grewal and Flavell, 1996). Estrogen deficiency increases the number of activated CD40L-expressing T cells that promote the expression of M-CSF and RANKL by stromal cells and down-regulates the production of OPG. The net result is a significant increase in the rate of osteoclastogenesis (Li et al., 2011; Yokoyama et al., 2011). This mechanism was also described in bone loss due to increased PTH levels (Bedi et al., 2010; Gao et al., 2008).

Recently Th17 cells has been implicated in ovariectomy induced bone loss, these cells increased after ovariectomy due to the up regulation of STAT3, ROR- γ t and ROR- α and down regulation of Foxp3 which antagonizes Th17 cell differentiation. Th17 stimulates osteoclastogenesis through IL-17 production (Abdul et al., 2012), this effect is reversed by treatment with estradiol. IL-17 acts on osteoblasts reducing bone matrix mineralization and increasing their production of pro-osteoclastogenic cytokines as TNF- α , IL-6 and RANKL, these effects are antagonized by estradiol.

Activated T cells have also been suggested to inhibit osteoclastogenesis by diverting early OC precursors towards dendritic cells differentiation (Grcevic et al., 2006). Indeed T cells have the capacity to generate both osteoclastogenic cytokines such as RANKL and TNF α (D'Amelio et al., 2008), as well as anti-osteoclastogenic factors such as IL-4. It has also been suggested that the effects of activated T cells on osteoclastogenesis in vitro depends on the manner in which they are activated (Wyzga et al., 2004). The net effect of T cells on OCs formation may consequently represent the prevailing balance of anti and pro-osteoclastogenic T cell cytokine secretion. However in humans T cells seems to be pro-osteoclastogenic in different diseases including during estrogen deficiency (Colucci et al., 2004; D'Amelio et al., 2008; Giuliani et al., 2002; Oostlander et al., 2012; Roato et al., 2005; Roato et al., 2006; Roato et al., 2010; Zheng et al., 1997).

Taken together these observations demonstrate the causal relation among estrogen deprivation, T cell activation, increased cytokines production, and bone demineralization (Figure 1).

Estrogen deficiency, B cells and the regulation of bone turnover.

B cells are recognized for their capacity to produce antibodies, and for their role as professional antigen presenting cells. However, B cells have recently been directly implicated in the regulation of bone resorption as they represent a major source of OPG and, under certain conditions, could produce RANKL.

Recent data have shown that B cells are the dominant producers of OPG in the bone microenvironment in vivo (Li et al., 2007). In fact B cell KO mice has an osteoporotic phenotype with enhanced osteoclastic bone resorption (Li et al., 2007).

Reconstitution of young B cell KO mice with B cells by means of adoptive transfer, completely rescued mice from development of osteoporosis, by normalizing OPG production (Li et al., 2007).

Human and animals B cell OPG production can be significantly up-regulated by the activation of CD40 (Li et al., 2007; Yun et al., 1998). In line with these data both CD40 and CD40L KO mice displayed an osteoporotic phenotype and a significant deficiency in bone marrow OPG concentrations (Li et al., 2007).

Thus the emerging data suggests that the B lineage, rather than the osteoblast lineage, is likely the major source of OPG in the bone microenvironment and that T cell signaling to B cells, through the co-stimulatory molecules CD40L and CD40 play an important role in regulating basal OCs formation and in regulating bone homeostasis.

On the other hand it has been recently demonstrated that activated B cells over-express RANKL, contributing to bone resorption (Han X et al., 2009; Yeo et al., 2011), and that

ovariectomy in mice increases the number of RANKL-expressing B lymphocytes in the bone marrow (Kanematsu et al., 2000), (Figure 1).

A recent paper shows that mice lacking RANKL in B cells were partially protected from the ovariectomy-induced loss of cancellous bone (Onal et al., 2012). The role of B-lymphocytes has also been evaluated in disease characterized by focal bone loss as in periodontal inflammation (Han X et al., 2009; Han X et al., 2013) and rheumatoid arthritis (Yeo et al., 2011). In rheumatoid arthritis a recent paper suggest that B cells depletion ameliorates suppress bone turnover (Wheater et al., 2011).

Taken together these data suggest that B-lymphocyte involvement in the adaptive immune response contributes to bone resorption by up-regulating of RANKL expression through Toll like receptor pathways and align with the known ability of T cells to produce RANKL in presence of immune stimulus and to increase osteoclastogenesis.

The involvement of T and B cells in the control of bone turnover may provide a novel explanation for the propensity for osteopenia and osteoporosis development in numerous pathological conditions in which altered immune function or immunodeficiency in B cells and/or T cells results. Such conditions include HIV infection, solid organ and bone marrow transplantation, multiple myeloma in which normal B cells are significantly depleted, ageing, and patients treated with immunosuppressive agents such as glucocorticoids.

Conclusions.

In the last decay remarkable progress has been in exploiting the relation between immune system and bone loss. The main data were obtained in animal models, but in the recent years also interesting data on humans seems to confirm the hypothesis of a key role of the deregulation of immune cells in post-menopausal bone loss.

Literature data highlights the relationship among immune system, bone and estrogen withdrawal however a number of questions remain to be answered, such as the mechanisms by which these systems cross-regulate.

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Figure legend.

Figure 1. The cartoon represents interaction between estrogen deficiency, immune system and bone cells.

